For Research Use Only Not for Diagnostic Use

Rabbit Anti-*Haemophilus influenzae* type b IgG ELISA Assay

Catalog# IM-218

Introduction

Haemophilus influenzae type b can cause bacteremia, pneumonia, and acute bacterial meningitis in infants and young children. Vaccines have been developed with *Haemophilus influenzae* type b polysaccharide conjugated to a protein to protect from infection. Antibodies should be detectable in animals post vaccination.

Principle of the Assay

Microtiter wells coated with *Haemophilus influenzae* type b oligosaccharide-conjugated to human serum abumin (HbOHA) are exposed to test specimens, which may contain Rabbit IgG reactive antibodies. After an incubation period, unbound components in the test sample are washed away. Specific bound Rabbit IgG react with an anti-Rabbit IgG conjugated with HRP during a second incubation period. Following a second wash cycle, specific bound enzyme conjugate is detected by reaction with the substrate solution, 2,2' Azino-di[3-ethyl-benzthiazolinesulfonate (ABTS). The assay is measured spectrophotometrically to indicate the level of Rabbit Anti-Haemophilus influenzae type b antibodies present in a sample.

Kit Presentation

Materials Supplied
The reagents supplied in this pack are for Research use only.

1	Coated microwell strips. Plastic microtiter wells coated with HbOHA in foil pouch with desiccant.	1 plate (96 wells)
2	Rabbit Anti-*Haemophilus influenzae* type b Positive Calibrator 10000 U/ml	0.4 mL
3	Sample Diluent	30 mL
4	Conjugate. Anti- Rabbit IgG conjugated to horseradish peroxidase enzyme containing 0.01% Bromonitrodioxane as preservative.	12 mL
5	Wash Buffer (20x concentrated). Tris buffered saline pH 7.8-8.0, containing 0.05% Tween 20. Must be diluted before use.	1 Bottle 60 mL
6	Substrate Solution. Ready to use. 2,2' Azino-di[3-ethyl-benzthiazoline-sulfonate] ABTS	12 mL
7	Stop Solution. 1.25% sodium fluoride	5 mL

Additional Requirements for Manual Processing
> Disposable tip micropipettes to deliver volumes of 5 µL, 10 µL, 25 µL, 100 µL and 200 µL (multichannel pipette preferred for dispensing reagents into microtiter plates).
> Distilled or deionized water.
> 37 (±1)º C incubator.
> Clean, disposable plastic/ glass test tubes, approximate capacities 5 mL and 10 mL.
> Range of standard, clean volumetric laboratory glassware consisting of, at least, 15 mL and 100 mL beakers, 1 L graduated cylinder,1 mL, 5 mL, and 10 mL glass pipettes.
> Absorbent paper towels.
> Automatic microtitration plate washer or laboratory wash bottle.
> Microtitration plate reader with 450 nm filter.
> Latex gloves, safety glasses and other appropriate protective garments.
> Biohazard infectious waste containers.
> Safety pipetting devices for 1 mL or larger pipettes.
> Timer.

Automatic, or Semi-automatic Processing

The Rabbit Anti-*Haemophilus influenzae* type b Assay may be used with a variety of automatic or semi-automatic processors/liquid handling systems. It is essential that any such system is qualified, before it is used routinely, by demonstrating that the Rabbit Anti-*Haemophilus influenzae* type b Assay results obtained using the automatic processor are equivalent to those obtained for the same specimens using the manual test method. Subsequently the automatic processor should be periodically re-qualified.

Storage and Stability

All reagents should be stored at 2-8°C , and should not be used beyond the expiration date on the label. Once opened, microtitration strips may be stored at 2-8°C until the expiration date on the label, provided that desiccated conditions are maintained. Unused strips should be returned to their original foil pouch along with the sachet of desiccant. Opened pouches should be securely resealed by folding over the open end and securing it with adhesive tape.

The working strength Wash Buffer should not be stored for longer than 3 weeks at 2-8°C. It is recommended that Wash buffer be freshly diluted before each assay. If the working strength buffer becomes visibly cloudy or develops precipitate during the 3 weeks, do not use it.

Indications of Deterioration
The Rabbit Anti-*Haemophilus influenzae* type b Assay may be considered to have deteriorated if:
1. The kit fails to meet the required criteria for a valid test (see Interpretation of Results).
2. Reagents becoming visibly cloudy or develop precipitate. *Note*: Concentrated Wash buffer, when cold, normally develops crystalline precipitates, which re-dissolve on heating at 37°C.
3. The Substrate Solution turns dark green. This is likely to be caused by contamination of the Substrate Solution.

Warnings and Precaution

Safety
1. The reagents supplied in this kit are for ***Research use only***.
2. Caution: All blood products should be treated as potentially infectious. Essential precautions can be summarized as follows**:**
   >do not pipette by mouth.
   >Wear disposable gloves during all specimen and assay manipulations.
   >Avoid use of sharp or pointed liquid handling devices, which may puncture skin.
   >Do not smoke, eat or drink in the laboratory work area.
   >Avoid splashing of liquid specimens and reagents and the formation of aerosols.
   >Wash hands thoroughly on completion of a manipulation.
   >The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Bio safety Level 2.
3. The Rabbit Anti-*Haemophilus influenzae* type b kits contain reagent systems, which are optimized and balanced for each kit lot. Do not interchange reagents from kits with different lot numbers. Do not interchange vial caps or stoppers either within or between kits.

4. The Substrate Solution and Stop Solution in this kit contain ingredients that can irritate the skin and cause eye damage. Handle them with care and wear suitable protective clothing and eye/face protection. In case of contact with skin or eyes, immediately flush the affected area with plenty of water. For eyes, obtain medical attention.

Procedural

1. This kit should be used in strict accordance with the instructions in the Package Insert.
2. Do not use Rabbit Anti-*Haemophilus influenzae* type b Assay kits after the expiration date printed on the outer carton label.
3. Do not cross contaminate reagents. Always use fresh pipette tips when drawing from stock reagent bottles.
4. Always use clean, preferably disposable, glassware for all reagent preparation.
5. Allow foil bags to warm to room temperature before opening. This avoids condensation on the inner surface of the bag, which may contribute to a deterioration of coated strips intended for future use.
6. Reagents should be dispensed with the tip of the micropipettes touching the side of the well at a point about mid-section. Follow manufacturer's recommendations for automatic processors.
7. Always keep the upper surface of the microtiter strips free from excess fluid droplets. Reagents and buffer over-spill should be blotted dry on completion of the manipulation.
8. Do not allow the wells to completely dry during an assay.
9. Disposal or decontamination of fluid in the waste reservoir from either the plate washer or trap for vacuum line in the manual system should be in accordance with guidelines set forth in the Department of Labor, Occupational Safety and Health Administration, occupational exposure to blood-borne pathogens; final rule (29 CFR 1910,1030) FEDERAL REGISTER, pp. 64176-84177,12/6/91.
10. Automatic or semi-automatic ELISA processors or liquid handling systems should be qualified specifically for use with Rabbit Anti-*Haemophilus influenzae* type b Assay by demonstration of equivalence to the manual processing methods.
11. Consistent with good laboratory practice, it is recommended that all pipetting devices (manual or automatic), timers and thermometers are regularly calibrated according to the manufacturer's instructions.
12 Care must be taken to ensure that specimens are dispensed correctly to each test well. If a specimen is inadvertently not added to a well, the result for that well will be non-reactive, regardless of the actual result of the specimen.

Method of Use

Specimen Collection and Storage

Obtain blood and allow clot to form. Remove serum from clot. Insoluble materials should be removed by centrifugation. Remove the serum aseptically. Serum samples should be refrigerated as soon as possible after collection. If not assayed within 48 hours, the samples should be aliquotted and frozen. Avoid repeated freezing/thawing of samples. Samples should not contain sodium azide.

Wash Cycle

Efficient washing to remove un-complexed components is a fundamental requirement of enzyme immunoassay procedures. The Rabbit Anti-*Haemophilus influenzae* type b assay utilises two standard five-wash cycles. Automatic plate washers may be used provided they meet the following criteria:

1 All wells are completely aspirated.
2. All wells are filled to the rim (350 µL) during the rinse cycle.
3. Wash buffer is dispensed at a good flow rate.
4. The plate washer must be well maintained to prevent contamination from previous use. Manufacturer's cleaning procedures must be followed diligently

For each wash cycle the machine should be set to five consecutive washes. On completion of the cycle, invert the microtitration plate and tap firmly on absorbent paper towels. Check for any residual wash buffer in the wells and blot dry the upper surface of the wells with a paper towel.

Alternatively, the following manual system may be employed:

1. Aspirate well contents using a vacuum line fitted with a trap.
2. Fill all wells to the brim with wash buffer dispensed from a squeeze-type laboratory wash bottle.
3. Aspirate all wells.
4. Repeat steps 2 and 3, four times for a total of five cycles.
5. Invert the microtiter plate and tap firmly on absorbent paper towels.

Preparation for the Assay

1. *Positive Anti-Haemophilus influenzae type b Calibrator 10000 U/ml*

a. Prepare working strength calibrator by diluting 50ul of the positive Anti-*Haemophilus influenzae* type b calibrator into 450 ul (1:10 dilution) of sample diluent. This will give a final concentration of 1000 U/ml.

b. Prepare six serial two fold dilutions (250ul standard with 250ul sample diluent) to prepare 500 U/ml, 250 U/ml, 125 U/ml, 62.5 U/ml, 31.2 U/ml and 15.6 U/ml calibrators using the sample diluent. Each calibrator plus a sample diluent (0 U/ml) should be run in duplicate.

2. *Wash Buffer*

Prepare working-strength Wash buffer by diluting 1 part concentrate with 19 parts of distilled or de-ionized water. If a kit is likely to be utilized over a period in excess of 4 weeks, then it is recommended that only enough stock concentrate be diluted sufficient for immediate needs. Each row of 8 wells may be adequately washed with 150 mL of working strength buffer.

Assay Procedure

1 Allow all reagents to reach room temperature (18-25°C).
2 Each Rabbit serum to be tested should be diluted to a 1:50 dilution. Dilute the serum 1:50 in Sample Diluent. For example: add 5 ul of serum sample to 245 ul of 1X Sample Diluent. If not assayed immediately, diluted samples should be stored at -20°C or below.
3 Select sufficient microtiter well strips to accommodate all test specimens and the 7 calibrators run in duplicate (including sample diluent). Fit the strips into the holding frame. Label wells according to specimen identity using the letter/number cross-reference system molded into the plastic frame.
4 Dispense 100 µL of each specimen, calibrator and sample diluent into appropriate wells. **Note: All standards and samples should be tested in duplicate.**
5 Incubate at 37(±1)°C for 45 (±5) minutes.
6 Aspirate the contents of the wells and wash the microtiter plate as described in the Rinse Cycle section.
7 Pipette 100 µL of anti-IgG conjugate into each well and incubate at 37(±1)°C for 45 (±5) minutes.
8 Aspirate the conjugate from the wells and wash the microtiter plate as described in the Wash section.
9 Without delay, dispense 100 µL Substrate Solution into each well. A multichannel pipette should be used for best results. Leave at room temperature (18-25°C) protected from direct sunlight, for 30 (±2) minutes.
10 If the plate is not read immediately, pipette 25 ul of Stop Solution into each test well. Within 15 minutes, read the plate at 405 nm using a microtiter plate reader blanked on the sample diluent well. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.

Interpretation of Results

The following criteria should be met for a valid assay:

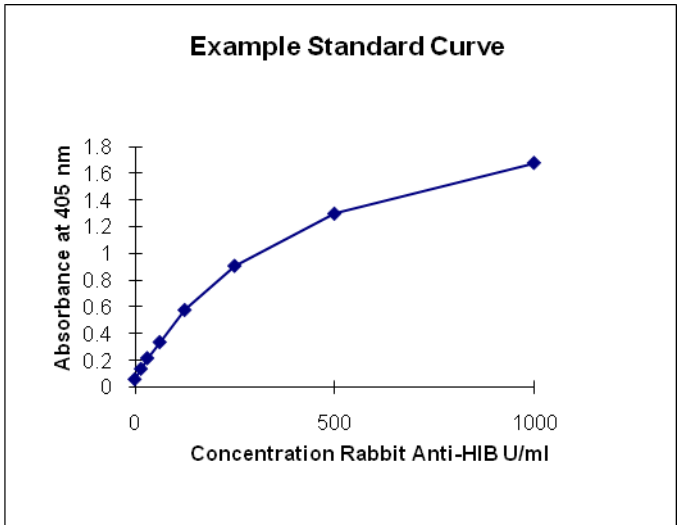
The 0 U/ml standard (sample diluent) should be ≤ 0.10
The 1000 U/ml standard should be ≥0.60

Quantitative Analysis

Manual Method: The calibration curve can be constructed manually on linear graph paper.
1. Calculate the mean absorbance for each standard.
2. Plot the mean absorbance on the y-axis versus the concentration of the standard on the x-axis. Connect the points to produce a point to point curve. Do not force the line to be linear.
3. The concentration of the specimens can be found directly from the standard curve. Please note that the standard curve has been standardized to the 1:50 serum dilution. The final U/ml can be read directly from the curve with no factor for dilution if the sample was assayed at a 1:50 dilution.

Table 1. Example Data at 405nm.

Sample	405 nm abs.	Mean abs.	U/mL
Sample Diluent (0 U/mL)	0.06		
"	0.06	0.06	
Standard 7 (15.6 U/mL)	0.13		
"	0.15	0.14	
Standard 6 (31.2 U/mL)	0.22		
"	0.22	0.22	
Standard 5 (62.5 U/mL)	0.32		
"	0.36	0.34	
Standard 4 (125 U/mL)	0.56		
"	0.60	0.58	
Standard 3 (250 U/mL)	0.88		
"	0.94	0.91	
Standard 2 (500 U/mL)	1.26		
"	1.34	1.30	
Standard 1 (1000 U/mL)	1.64		
"	1.72	1.68	
Specimen #1	0.42		
"	0.44	0.43	86
Specimen #2	0.67		
"	0.73	0.70	221



Note: The above standard curve is only an example and should not be used to generate any results.

Computer-Assisted Method: Computer assisted data reduction may be used to create the standard curve. Software providing a point to point curve fitting routine provides acceptable results.

Procedure for Samples with Rabbit Anti-*Haemophilus influenzae* type b assay values greater than the highest standard.

In order to obtain accurate results for samples with Rabbit Anti-*Haemophilus influenzae* type b assay values greater than the highest standard it is necessary to dilute and re-test the sample. Diluting the serum specimen 10-fold is recommended. For example: Make a 10-fold dilution by adding 0.1 mL of the initial specimen dilution (1:50) to 0.90 mL of sample diluent. Mix thoroughly and repeat the assay according to the Assay Procedure. Multiply the results by 10 to determine the correct Rabbit Anti-*Haemophilus influenzae* type b assay values in the sample.

Limitations of Use

1. Assay values determined using assays from different manufacturers or different methods may not be used interchangeably. **PLEASE NOTE:** There is not a international standard for Rabbit Anti-*Haemophilus influenzae* type b, therefore the units are assigned arbitrarily. Units between manufactures may not be the same.
2. Samples with very high Rabbit Anti-*Haemophilus influenzae* type b assay values levels may exhibit in a prozone effect.
3. The assay cannot be used to quantitate samples with Rabbit Anti-*Haemophilus influenzae* type b assay values greater than the highest standard without further serial dilution of the samples. See the Interpretation of Results section for directions on testing such samples.
4. The performance characteristics have not been established for any matrices other than serum.

Contact Information:

Express Biotech International
503 Gateway Drive West
Thurmont, MD 21788 USA
Tel: 301-228-2444
Fax: 301-560-6570
www.xpressbio.com
info@xpressbio.com